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(54) Title: RECOMBINANT MHC MOLECULES USEFUL FOR MANIPULATION OF ANTIGEN-SPECIFIC T-CELLS

(57) Abstract

Two-domain MHC polypeptides useful for manipulation of antigen-specific T-cells are disclosed. These polypeptides include MHC class II-based molecules that comprise covalently linked $\beta 1$ and $\alpha 1$ domains, and MHC class I-based molecules that comprise covalently linked $\alpha 1$ and $\alpha 2$ domains. These polypeptides may also include covalently linked antigenic determinants, toxic moieties, and/or detectable labels. The disclosed polypeptides can be used to target antigen-specific T-cells, and are useful, among other things, to detect and purify antigen-specific T-cells, to induce or activate T-cells, and to treat conditions mediated by antigen-specific T-cells.

RECOMBINANT MHC MOLECULES USEFUL FOR MANIPULATION OF ANTIGEN-SPECIFIC T-CELLS

Priority Claim

This application claims priority from co-pending U.S. provisional patent applications serial numbers 60/064,552 and 60/064,555, filed September 16, 1997 and October 10, 1997, respectively, which are incorporated herein by reference.

Background of the Invention

The initiation of an immune response against a specific antigen in mammals is brought about by the presentation of that antigen to T-cells. An antigen is presented to T-cells in the context of a major histocompatibility (MHC) complex. MHC complexes are located on the surface of antigen presenting cells (APCs); the 3-dimensional structure of MHCs includes a groove or cleft into which the presented antigen fits. When an appropriate receptor on a T-cell interacts with the MHC/antigen complex on an APC in the presence of necessary co-stimulatory signals, the T-cell is stimulated, triggering various aspects of the well characterized cascade of immune system activation events, including induction of cytotoxic T-cell function, induction of B-cell function and stimulation of cytokine production.

There are two basic classes of MHC molecules in mammals, MHC class I and MHC II. Both classes are large protein complexes formed by association of two separate proteins. Each class includes trans-membrane domains that anchor the complex into the cell membrane. MHC class I molecules are formed from two non-covalently associated proteins, the α chain and β2-microglobulin. The α chain comprises three distinct domains, α1, α2 and α3. The three dimensional structure of the α1 and α2 domains forms the groove into which antigens fit for presentation to T-cells. The α3 domain is a trans-membrane Ig-fold like domain that anchors the α chain into the cell membrane of the APC. MHC class I complexes, when associated with antigen (and in the presence of appropriate co-stimulatory signals) stimulate CD8 cytotoxic T-cells, which function to kill any cell which they specifically recognize.

The two proteins which associate non-covalently to form MHC class II molecules are termed the α and β chains. The α chain comprises α1 and α2 domains, and the β chain comprises β1 and β2 domains. The cleft into which the antigen fits is formed by the interaction of the α1 and β1 domains. The α2 and β2 domains are trans-membrane Ig-fold like domains that anchors the α and β chains into the cell membrane of the APC. MHC class II complexes, when associated with antigen (and in the presence of appropriate co-stimulatory signals) stimulate CD4 T-cells. The primary functions of CD4 T-cells are to initiate the inflammatory response and to regulate other cells in the immune system.

The genes encoding the various proteins that constitute the MHC complexes have been extensively studied in humans and other mammals. In humans, MHC molecules (with the exception

(Science 274, 94-96 (1996) and U.S. patent No. 5,635,363) have proposed the use of large, covalently linked multimeric structures of MHC/antigen complexes to stabilize this interaction by simultaneously binding to multiple T-cell receptors on a target T-cell.

Although the concept of using isolated MHC/antigen complexes in therapeutic and diagnostic applications holds great promise, a major drawback to the various methods reported to date is that the complexes are large and consequently difficult to produce and to work with. While the complexes can be isolated from lymphocytes by detergent extraction, such procedures are inefficient and yield only small amounts of protein. The cloning of the genes encoding the various MHC complex subunits has facilitated the production of large quantities of the individual subunits through expression in prokaryotic cells, but the assembly of the individual subunits into MHC complexes having the appropriate conformational structure has proven difficult.

Summary Of The Invention

This invention is founded on the discovery that mammalian MHC function can be mimicked through the use of recombinant polypeptides that include only those domains of MHC molecules that define the antigen binding cleft. These molecules are useful to detect, quantify and purify antigen-specific T-cells. The molecules provided herein may also be used in clinical and laboratory applications to detect, quantify and purify antigen-specific T-cells, induce anergy in T-cells, as well as to stimulate T-cells, and to treat diseases mediated by antigen-specific T-cells.

By way of example, while Altman et al. (U.S. patent No. 5,635,363) contemplate the use of multimers of MHC class II complexes comprising α1, α2, β1 and β2 domains and associated peptide antigens, to bind to and purify antigen-specific T-cells from a mixture, the present inventors have discovered that such antigen-specific T-cell binding can be accomplished with a much simpler monomeric molecule comprising, in the case of class II MHC molecules, only the α1 and β1 domains in covalent linkage (and in association with an antigenic determinant). For convenience, such MHC class II polypeptides are hereinafter referred to as "β1α1". Equivalent molecules derived from MHC class I molecules are also provided by this invention. Such molecules comprise the α1 and α2 domains of class I molecules in covalent linkage and in association with an antigenic determinant. Such MHC class I polypeptides are referred to as "α1α2". These two domain molecules may be readily produced by recombinant expression in prokaryotic or eukaryotic cells, and readily purified in large quantities. Moreover, these molecules may easily be loaded with any desired peptide antigen, making production of a repertoire of MHC molecules with different T-cell specificities a simple task.

It is shown that, despite lacking the trans-membrane Ig fold domains that are part of intact MHC molecule, these two domain MHC molecules refold in a manner that is structurally analogous to "whole" MHC molecules, and bind peptide antigens to form stable MHC/antigen complexes. Moreover, these two domain MHC/epitope complexes bind T-cells in an epitope-specific manner, and inhibit epitope-specific T-cell proliferation *in vitro*. In addition, administration of β1α1

cells having a particular antigen specificity. Alternatively, the molecules may also be used to induce anergy in such T-cells.

The two domain molecules may also be used *in vivo* to target specified antigen-specific T-cells. By way of example, a β1α1 molecule loaded with a portion of myelin basic protein (MBP) and administered to patients suffering from multiple sclerosis may be used to induce anergy in MBP-specific T-cells, thus alleviating the disease symptoms. Alternatively, such molecules may be conjugated with a toxic moiety to more directly kill the disease-causing T-cells.

These and other aspects of the invention are described in more detail in the following sections.

Brief Description of the Drawings

Fig. 1A shows the sequences of the prototypical β1α1 cassette without an antigen coding region. Unique NcoI, PstI, and XhoI restriction sites are in **bold.** The end of the β1 domain and start of the α1 domain are indicated. Fig. 1B shows the sequence of an in-frame antigenic peptide/linker insertion sequence that can be incorporated into the expression cassette at the insertion site shown (▾) in Fig. 1A. This sequence includes the rat MBP 72-89 antigen, a flexible linker with an embedded thrombin cleavage site, and a unique SpeI restriction site that can be used for facile exchange of the antigen coding region. Example 2 below discusses the use of the equivalent peptide from Guinea pig, which has a serine in place of the threonine residue in the MBP 72-89 sequence. Figs. 1C and 1D show exemplary Nco1/SpeI fragments that can be inserted into the expression cassette in place of the MBP-72-89 antigen coding region. Fig. 1C includes the MBP 55-69 antigen, Fig. 1D includes the CM-2 antigen.

Figs. 2A and B show the structure-based design of the ß1α1 molecule. A. Rat class II RT1.B, loaded with the encephalitogenic MBP-69-89 peptide. B. The single-chain ß1α1 molecule, loaded with MBP-69-89.

Figs. 3 A and B show direct detection of antigen-specific β1α1/polypeptide molecules binding rat T cells. The A1 T cell hybridoma (BV8S2 TCR+) and the CM-2 cell line (BV8S2 TCR-) were incubated 17 hours at 4°C with various β1α1 constructs, washed, stained for 15 min with OX6-PE (α-RT1.B) or a PE-isotype control and then analyzed by FACS. Background expression of I-A on the CM-2 line was blocked with unlabeled OX-6. A. Histogram showing staining of the A1 hybridoma. B. Histogram showing staining of the CM-2 cell line.

Fig. 4 is a graph showing binding of A488 conjugated β1α1/polypeptide molecules to rat BV8S2 TCR. β1α1 molecules were conjugated with Alexa-488 dye, loaded with MBP-69-89, incubated with the A1 T cell hybridomas (BV8S2 TCR+) for 3 hours at 4°C and then analyzed by FACS. A488-β1α1(empty) and A488-β1α1/MBP-69-89, as indicated.

Fig. 5 is a bar graph showing that the ß1α1/MBP-69-89 complex blocks antigen specific proliferation in an IL-2 reversible manner. Short-term T cell lines selected with MBP-69-89 peptide from lymph node cells from rats immunized 12 days earlier with Gp-MBP/CFA were pre-treated for 24 hours with ß1α1 constructs, washed, and then used in proliferation assays in which the cells were

Fig. 11 shows the amino acid sequences of exemplary α1 and α2 domains derived from human MHC class I B*5301.

Sequence Listing

The sequence listing appended hereto includes sequences as follows:

Seq. I.D. No. 1: the nucleic acid of a single chain β1α1 expression cassette.

Seq. I.D. No. 2: the amino acid sequence encoded by the construct shown in Seq. I.D. No. 1.

Seq. I. D No. 3: the nucleic acid sequence of an antigen/linker insert suitable for insertion into the expression cassette shown in Seq. I.D. No. 1.

Seq. I.D. No. 4: the amino acid sequence encoded by the sequence shown in Seq. I.D. no. 3.

Seq. I.D. Nos. 5 and 7: alternative antigen encoding sequences for the expression cassette and, Seq. I.D. Nos. 6 and 8, the antigen sequences encoded by the sequences shown in Seq. I.D. Nos. 5 and 7, respectively.

Seq. I.D. Nos. 9 - 20 and 28-29 show PCR primers use to amplify components of the β1α1 expression cassette.

Seq. I.D. No. 21 shows the exemplary α1 and α2 domains depicted in Fig. 11.

Seq. I.D. Nos. 22-24 show the exemplary β1 and α1 domains depicted in Fig. 10.

Seq. I.D. Nos. 25-27, and 30 show peptides sequences used in various aspects of the invention.

Detailed Description of the Invention

1. Definitions

In order to facilitate review of the various embodiments of the invention, the following definitions of terms and explanations of abbreviations are provided:

Isolated: An "isolated" nucleic acid has been substantially separated or purified away from other nucleic acid sequences in the cell of the organism in which the nucleic acid naturally occurs, i.e., other chromosomal and extrachromosomal DNA and RNA. The term "isolated" thus encompasses nucleic acids purified by standard nucleic acid purification methods. The term also embraces nucleic acids prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids.

cDNA (complementary DNA): a piece of DNA lacking internal, non-coding segments (introns) and regulatory sequences which determine transcription. cDNA is synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

ORF (open reading frame): a series of nucleotide triplets (codons) coding for amino acids without any termination codons. These sequences are usually translatable into a polypeptide.

polypeptide is such that the carboxy terminus of the β1 domain is covalently linked to the amino terminus of the α1 domain.

β1α1 gene: a recombinant nucleic acid sequence including a promoter region operably linked to a nucleic acid sequence encoding a β1α1 polypeptide.

α1α2 polypeptide: a polypeptide comprising the α1 and α2 domains of a MHC class I molecule in covalent linkage. The orientation of such a polypeptide is such that the carboxy terminus of the α1 domain is covalently linked to the amino terminus of the α2 domain. An α1α2 polypeptide comprises less than the whole class I α chain, and usually omits most or all of the α3 domain of the α chain.

α1α2 gene: a recombinant nucleic acid sequence including a promoter region operably linked to a nucleic acid sequence encoding an α1α2 polypeptide.

Domain: a domain of a polypeptide or protein is a discrete part of an amino acid sequence that can be equated with a particular function. For example, the α and β polypeptides that constitute a MHC class II molecule are each recognized as having two domains, α1, α2 and β1, β2, respectively. Similarly, the α chain of MHC class I molecules is recognized as having three domains, α1, α2 and α3. The various domains in each of these molecules are typically joined by linking amino acid sequences. When selecting the sequence of a particular domain for inclusion in a recombinant molecule, it is preferable that the entire domain be included; to ensure that this is done, the domain sequence may be extended to include part of the linker, or even part of the adjacent domain. For example, when selecting the α1 domain of HLA-DR A, the selected sequence will generally extend from amino acid residue number 1 of the α chain, through the entire α1 domain and will include include all or part of the linker sequence located at about amino acid residues 76-90 (at the carboxy terminus of the α1 domain, between the α1 and α2 domains). However, the precise number of amino acids in the various MHC molecule domains varies depending on the species of mammal, as well as between classes of genes within a species. Rather than a precise structural definition based on the number of amino acids, it is the maintenance of domain function that is important when selecting the amino acid sequence of a particular domain. Moreover, one of skill in the art will appreciate that domain function may also be maintained if somewhat less than the entire amino acid sequence of the selected domain is utilized. For example, a number of amino acids at either the amino or carboxy terminii of the α1 domain may be omitted without affecting domain function. Typically however, the number of amino acids omitted from either terminus of the domain sequence will be no greater than 10, and more typically no greater than 5. The functional activity of a particular selected domain may be assessed in the context of the two-domain MHC polypeptides provided by this invention (i.e., the class II β1α1 or class I α1α2 polypeptides) using the antigen-specific T-cell proliferation assay as described in detail below. For example, to test a particular β1 domain, it will be linked to a functional α1 domain so as to produce a β1α1 molecule and then tested in the described assay. A biologically active β1α1 or α1α2 polypeptide will inhibit antigen-specific T cell proliferation by at least about 50%, thus indicating that the component domains are functional. Typically, such

which are generally between 2 and 25 amino acids in length, are well known in the art and include the glycine(4)-serine spacer (GGGGS x3) described by Chaudhary et al. (1989).

Recombinant MHC class I α1α2 polypeptides according to the present invention include a covalent linkage joining the carboxy terminus of the α1 domain to the amino terminus of the α2 domain. The α1 and α2 domains of native MHC class I α chains are typically covalently linked in this orientation by an amino acid linker sequence. This native linker sequence may be maintained in the recombinant constructs; alternatively, a recombinant linker sequence may be introduced between the α1 and α2 domains (either in place of or in addition to the native linker sequence).

Additional definitions of terms commonly used in molecular genetics can be found in Benjamin Lewin, *Genes V* published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew et al (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

The following sections provide detailed guidance on the design, expression and uses of the recombinant MHC molecules of the invention. Unless otherwise stated, standard molecular biology, biochemistry and immunology methods are used in the present invention unless otherwise described. Such standard methods are described in Sambrook et al. (1989), Ausubel et al (1987), Innis et al. (1990) and Harlow and Lane (1988). The following U.S. patents which relate to conventional formulations of MHC molecules and their uses are incorporated herein by reference to provide additional background and technical information relevant to the present invention: 5,130,297; 5,194,425; 5,260,422; 5,284,935; 5,468,481; 5,595,881; 5,635,363; 5,734,023.

2. Design Of Recombinant MHC Class II β1α1 Molecules

The amino acid sequences of mammalian MHC class II α and β chain proteins, as well as nucleic acids encoding these proteins, are well known in the art and available from numerous sources including GenBank. Exemplary sequences are provided in Auffray et al. (1984) (human HLA DQ α); Larhammar et al. (1983) (human HLA DQ β); Das et al. (1983) (human HLA DR α); Tonnelle et al. (1985) (human HLA DR β); Lawrance et al. (1985) (human HLA DP α); Kelly et al. (1985) (human HLA DP β); Syha et al. (1989) (rat RT1.B α); Syha-Jedelhauser et al. (1991) (rat RT1.B β); Benoist et al. (1983) (mouse I-A α); Estess et al. (1986) (mouse I-A β).

The recombinant MHC class II molecules of the present invention comprise the β1 domain of the MHC class II β chain covalently linked to the α1 domain of the MHC class II α chain. The β1 and α1 domains are well defined in mammalian MHC class II proteins. Typically, the α1 domain is regarded as comprising about residues 1-90 of the mature α chain. The native peptide linker region between the α1 and α2 domains of the MHC class II protein spans from about amino acid 76 to about amino acid 93 of the α chain, depending on the particular α chain under consideration. Thus, an α1 domain may include about amino acid residues 1-90 of the α chain, but one of skill in the art will recognize that the C-terminal cut-off of this domain is not necessarily precisely defined, and, for

sequence may conveniently be provided by designing the PCR primers to encode the linker sequence. Thus, in the example described above, the linker sequence may be encoded by one of the B2 or A1 primers, or a combination of each of these primers.

3. **Design Of Recombinant MHC Class I α1α2 Molecules**

The amino acid sequences of mammalian MHC class I α chain proteins, as well as nucleic acids encoding these proteins, are well known in the art and available from numerous sources including GenBank. Exemplary sequences are provided in Browning et al. (1995) (human HLA-A); Kato et al. (1993) (human HLA-B); Steinle et al. (1992) (human HLA-C); Walter et al. (1995) (rat Ia); Walter et al. (1994) (rat Ib); Kress et al. (1983) (mouse H-2-K); Schepart et al. (1986) (mouse H-2-D); and Moore et al. (1982) (mouse H-2-I).

The recombinant MHC class I molecules of the present invention comprise the α1 domain of the MHC class I α chain covalently linked to the α2 domain of the MHC class I α chain. These two domains are well defined in mammalian MHC class I proteins. Typically, the α1 domain is regarded as comprising about residues 1-90 of the mature α chain and the α2 chain as comprising about amino acid residues 90-180, although again, the cut-off points are not precisely defined and will vary between different MHC class I molecules. The boundary between the α2 and α3 domains of the MHC class I α protein typically occurs in the region of amino acids 179-183 of the mature α chain. The composition of the α1 and α2 domains may also vary outside of these parameters depending on the mammalian species and the particular α chain in question. One of skill in the art will appreciate that the precise numerical parameters of the amino acid sequence are much less important than the maintenance of domain function. An exemplary α1α2 molecule is depicted in Fig. 11.

The α1α2 construct may be most conveniently constructed by amplifying the reading frame encoding the dual-domain (α1 and α2) region between amino acid number 1 and amino acids 179-183, although one of skill in the art will appreciate that some variation in these end-points is possible. Such a molecule includes the native linker region between the α1 and α2 domains, but if desired that linker region may be removed and replaced with a synthetic linker peptide. The general considerations for amplifying and cloning the MHC class I α1 and α2 domains apply as discussed above in the context of the class II β1 and α1 domains.

4. **Genetic Linkage of of Antigenic Polypeptide to β1α1 and α1α2 Molecules**

The class II β1α1 and class I α1α2 polypeptides of the invention are generally used in conjunction with an antigenic peptide. Any antigenic peptide that is conventionally associated with class I or class II MHC molecules and recognized by a T-cell can be used for this purpose. Antigenic peptides from a number of sources have been characterized in detail, including antigenic peptides from honey bee venom allergens, dust mite allergens, toxins produced by bacteria (such as tetanus toxin) and human tissue antigens involved in autoimmune diseases. Detailed discussions of such

coding region with the selected restriction enzyme, (b) cleaving the MHC construct with the same restriction enzyme, and (c) ligating the antigen coding region into the MHC construct. In this manner, a large number of MHC-polypeptide constructs can be made and expressed in a short period of time.

An exemplary design of an expression cassette allowing simple exchange of antigenic peptides in the context of a β1α1 molecule is shown in Fig. 1. Fig 1A shows the nucleic acid sequence encoding a prototype β1α1 molecule derived from rat MHC class II RT1.B, without the presence of the antigenic peptide. The position of the insertion site for the peptide and linker between the 5th and 6th (serine and proline) residues of the β1 domain is indicated by a ▾symbol. In order to integrate the antigen coding region, a PCR primer comprising the sequence shown in Fig. 1B joined with additional bases from the Fig. 1A construct 3' of the insertion site is employed in conjunction with a PCR primer reading from the 3' end of the construct shown in Fig. 1A.) Amplification yields a product that includes the sequence shown in Fig. 1B integrated into the β1α1 construct (i.e., with the antigenic peptide and linker sequences positioned between the codons encoding the 5th and 6th amino acid residues of the β1α1 sequence). In the case illustrated, the antigenic peptide is the MBP-72-89 antigen.

Notably, the MBP-72-89 coding sequence is flanked by unique Nco I and Spe I restriction enzyme sites. These enzymes can be used to release the MBP-72-89 coding region and replace it with coding regions for other antigens, for example those illustrated in Figs. 1C and 1D.

The structure of the expressed β1α1 polypeptide with covalently attached antigen is illustrated in Fig. 2B; Fig. 2A shows the secondary structure of the complete RT1B molecule (including α1, α2, β1 and β2 domains).

Nucleic acid expression vectors including expression cassettes designed as explained above will be particularly useful for research purposes. Such vectors will typically include sequences encoding the dual domain MHC polypeptide (β1α1 or α1α2) with a unique restriction site provided towards the 5' terminus of the MHC coding region, such that a sequence encoding an antigenic polypeptide may be conveniently attached. Such vectors will also typically include a promoter operably linked to the 5' terminus of the MHC coding region to provide for high level expression of the sequences.

β1α1 and α1α2 molecules may also be expressed and purified without an attached peptide (as described in section 5 below), in which case they may be referred to as "empty". The empty MHC molecules may then be loaded with the selected peptide as described in section 6 below.

5. Expression and Purification of Recombinant β1α1 and α1α2 Molecules

In their most basic form, nucleic acids encoding the MHC polypeptides of the invention comprise first and second regions, having a structure A-B wherein, for class I molecules, region A encodes the class I α1 domain and region B encodes the class I α2 domain. For class II molecules, A encodes the class II β1 domain and B encodes the class II α1 domain. Where a linker sequence is

HeLa, *Spodoptera frugiperda*, and *Saccharomyces cerevisiae* may also be used to express the MHC polypeptides. Regulatory regions suitable for use in these cells include, for mammalian cells, viral promoters such as those from CMV, adenovirus and SV40, and for yeast cells, the promoter for 3-phosphoglycerate kinase and alcohol dehydrogenase.

The transfer of DNA into eukaryotic, in particular human or other mammalian cells, is now a conventional technique. The vectors are introduced into the recipient cells as pure DNA (transfection) by, for example, precipitation with calcium phosphate or strontium phosphate, electroporation, lipofection, DEAE dextran, microinjection, protoplast fusion, or microprojectile guns. Alternatively, the nucleic acid molecules can be introduced by infection with virus vectors. Systems are developed that use, for example, retroviruses, adenoviruses, or Herpes virus.

An MHC polypeptide produced in mammalian cells may be extracted following release of the protein into the supernatant and may be purified using an immunoaffinity column prepared using anti-MHC antibodies. Alternatively, the MHC polypeptide may be expressed as a chimeric protein with, for example, b-globin. Antibody to b-globin is thereafter used to purify the chimeric protein. Corresponding protease cleavage sites engineered between the b-globin gene and the nucleic acid sequence encoding the MHC polypeptide are then used to separate the two polypeptide fragments from one another after translation. One useful expression vector for generating b-globin chimeric proteins is pSG5 (Stratagene, La Jolla, CA).

Expression of the MHC polypeptides in prokaryotic cells will result in polypeptides that are not glycosylated. Glycosylation of the polypeptides at naturally occurring glycosylation target sites may be achieved by expression of the polypeptides in suitable eukaryotic expression systems, such as mammalian cells.

Purification of the expressed protein is generally performed in a basic solution (typically around pH 10) containing 6M urea. Folding of the purified protein is then achieved by dialysis against a buffered solution at neutral pH (typically phosphate buffered saline (PBS) at around pH 7.4).

6. Antigen Loading of Empty β1α1 and α1α2 Molecules

Where the β1α1 and I α1α2 molecules are expressed and purified in an empty form (i.e., without attached antigenic peptide), the antigenic peptide may be loaded into the molecules using standard methods. Methods for loading of antigenic peptides into MHC molecules is described in, for example, U.S. patent No. 5,468,481. Such methods include simple co-incubation of the purified MHC molecule with a purified preparation of the antigen.

By way of example, empty ß1a1 molecules (1mg/ml; 40uM) may be loaded by incubation with a 10-fold molar excess of peptide (1mg/ml; 400uM) at room temperature, for 24 hours. Thereafter, excess unbound peptide may be removed by dialysis against PBS at 4 °C for 24 hours. As is known in the art, peptide binding to ß1a1 can be quantified by silica gel thin layer chromatography (TLC) using radiolabeled peptide. Based on such quantification, the loading may be altered (e.g., by changing the molar excess of peptide or the time of incubation) to obtain the desired result.

Table 1.

Original Residue	Conservative Substitutions
Ala	ser
Asn	gln; his
Asp	glu
Cys	ser
Gln	asn
Glu	asp
Gly	pro
His	asn; gln
Ile	leu; val
Leu	ile; val
Lys	arg; gln; glu
Met	leu; ile
Phe	met; leu; tyr
Ser	thr
Thr	ser
Trp	tyr
Tyr	trp; phe
Val	ile; leu

More substantial changes in biological function or other features may be obtained by selecting substitutions that are less conservative than those in Table 1, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine. The effects of these amino acid substitutions or deletions or additions may be assessed through the use of the described T-cell proliferation assay.

At the nucleic acid level, one of skill in the art will appreciate that the naturally occurring nucleic acid sequences that encode class I and II MHC domains may be employed in the expression vectors, but that the invention is not limited to such sequences. Any sequence that encodes a functional MHC domain may be employed, and the nucleic acid sequence may be adapted to conform with the codon usage bias of the organism in which the sequence is to be expressed.

b. Incorporation of Detectable Markers

For certain in vivo and in vitro applications, the MHC molecules of the present invention may be conjugated with a detectable label. A wide range of detectable labels are known, including radionuclides (e.g., gamma-emitting sources such as indium-111), paramagnetic isotopes, fluorescent markers (e.g., fluorescein), enzymes (such as alkaline phosphatase), cofactors, chemiluminescent

this method of delivery. Additional possible methods of delivery include deep lung delivery by inhalation (Edwards et al., 1997; Service, 1997) and trans-dermal delivery (Mitragotri et al., 1996).

It is also contemplated that the MHC polypeptides of the present invention could be delivered to cells in the nucleic acid form and subsequently translated by the host cell. This could be done, for example through the use viral vectors or liposomes. Liposomes could also be used for direct delivery of the polypeptides.

The pharmaceutical compositions of the present invention may be administered by any means that achieve their intended purpose. Amounts and regimens for the administration of the selected MHC polypeptides will be determined by the attending clinician. Effective doses for therapeutic application will vary depending on the nature and severity of the condition to be treated, the particular MHC polypeptide selected, the age and condition of the patient and other clinical factors. Typically, the dose range will be from about 0.1 ug/kg body weight to about 100mg/kg body weight. Other suitable ranges include doses of from about 100 ug/kg to 1mg/kg body weight. The dosing schedule may vary from once a week to daily depending on a number of clinical factors, such as the subject's sensitivity to the protein. Examples of dosing schedules are 3 ug/kg administered twice a week, three times a week or daily; a dose of 7 ug/kg twice a week, three times a week or daily; a dose of 10 ug/kg twice a week, three times a week or daily; or a dose of 30 ug/kg twice a week, three times a week or daily.

8. Exemplary Applications of Recombinant β1α1 and α1α2 Molecules

The class II β1α1 and class I α1α2 polypeptides of the present invention are useful for a wide range of *in vitro* and *in vivo* applications,. Indeed, as a result of the biological activities of these polypeptides, they may be used in numerous application in place of either intact purified MHC molecules, or antigen presenting cells that express MHC molecules.

In vitro applications of the disclosed polypeptides include the detection, quantification and purification of antigen-specific T-cells. Methods for using various forms of MHC-derived complexes for these purposes are well known and are described in, for example, U.S. patent Nos. 5,635,363 and 5,595,881. For such applications, the disclosed polypeptides may be free in solution or may be attached to a solid support such as the surface of a plastic dish, a microtiter plate, a membrane, or beads. Typically, such surfaces are plastic, nylon or nitrocellulose. Polypeptides in free solution are useful for applications such as fluorescence activated sell sorting (FACS). For detection and quantification of antigen-specific T-cells, the polypeptides are preferably labeled with a detectable marker, such as a fluorescent marker.

The T-cells to be detected, quantified or otherwise manipulated are generally present in a biological sample removed from a patient. The biological sample is typically blood or lymph, but may also be tissue samples such as lymph nodes, tumors, joints etc. It will be appreciated that the precise details of the method used to manipulate the T-cells in the sample will depend on the type of manipulation to be performed and the physical form of both the biological sample and the MHC

forms of MHC polypeptides that may be used to treat these conditions and the methods used in those systems are equally useful with the MHC polypeptides of the present invention. Exemplary methodologies are described in U.S. patent Nos. 5,130,297, 5,284,935, 5,468,481, 5,734,023 and 5,194,425. By way of example, the MHC/peptide complexes may be administered to patients in order to induce anergy in self-reactive T-cell populations, or these T-cell populations may be treated by administration of MHC/peptide complexes conjugated with a toxic moiety. The disclosed molecules may also be used to boost immune response in certain conditions such as cancer and infectious diseases.

EXAMPLES

The following Examples illustrate certain aspects of the invention.

Example 1: **Cloning, Expression and In Vitro Folding of β1α1 Molecules**

A prototypical nucleic acid construct was produced that encoded a single polypeptide chain with the amino terminus of the MHC class II α1 domain genetically linked to the carboxyl terminus of the MHC class II β1 domain. The sequence of this prototypical construct, made from the rat RT1B α- and ß-chain cDNAs is shown in Fig. 1A (Seq. I.D. No. 1).

RT1B α1- and ß1-domain encoding cDNAs were prepared by PCR amplification of cloned RT1.B α- and ß-chain cDNA coding sequences (α6, ß118, respectively) obtained from Dr. Konrad Reske, Mainz, FRG (Syha et al., 1989; Syha-Jedelhauser et al., 1991). The primers used to generate ß1 were 5'-AATTCCTCGAGATGGCTCTGCAGACCCC-3' (XhoI 5' primer) (Seq. I.D. No. 9); 5'-TCTTGACCTCCAAGCCGCCGCAGGGAGGTG-3' (3' ligation primer) (Seq. I.D. No. 10). The primers used to generate α1 were 5'-CGGCGGCTTGGAGGTCAAGACGACATTGAGG-3' (5' ligation primer) (Seq. I.D. No. 11); 5'-GCCTCGGTACCTTAGTTGACAGCTTGGGTTGAATTTG-3' (KpnI 3' primer) (Seq. I.D. No. 12). Additional primers used were 5'-CAGGGACCATGGGCAGAGACTCCCCA-3' (NcoI 5' primer) (Seq. I.D. No. 13); and 5'-GCCTCCTCGAGTTAGTTGACAGCTTGGGTT-3' (XhoI 3' primer) (Seq. I.D. No. 14). Step one involved production of cDNAs encoding the ß1 and α1 domains. PCR was conducted with Taq polymerase (Promega, Madison, WI) through 28 cycles of denaturation at 94.5°C for 20 seconds, annealing at 55°C for 1.5 minutes and extension at 72°C for 1.5 minutes, using ß118 as template and the XhoI 5' primer and 3' ligation primer as primers and α6 cDNA as template and the 5' ligation primer and KpnI 3' primer. PCR products were isolated by agarose gel electrophoresis and purified using Gene-Clean (Bio 101, Inc., La Jolla, CA).

In step two, these products were mixed together without additional primers and heat denaturated at 94.5°C for 5 minutes followed by 2 cycles of denaturation at 94.5°C for 1 minute, annealing at 60°C for 2 minutes and extension at 72°C for 5 minutes. In step three, the annealed, extended product was heat denaturated at 94.5°C for 5 minutes and subjected to 26 cycles of denaturation at 94.5°C for 20 seconds, annealing at 60°C for 1 minute and extension at 72°C for 1

plasmid encoding the ß1a1/Gp-MBP-72-89 covalent construct. The primers used to generate the CM-2/linker cartridge were 5'-TATTACCATGGG CAGAGACTCCAAACTGGAACTGCAGTCCGCTCTGGAAGAAGCTGAAGCTT CCCTGGAACACGGAGGTGGAGGCTCACTAGTGCCCC-3' (5' CM-2 primer) (Seq. I.D. No. 19) and 5'-GGGGCACTAGTGAGCCTCCACCTCCGTGTTCCAGGGAAG CTTCAGCTTCTTCCAGAGCGGACTGCAGTTCCAGTTTGGAGTCTCTGCCCATGGTAATA-3' (3' CM-2 primer) (Seq. I.D. No. 20). These were gel purified, annealed and then cut with NcoI and XhoI for ligation into ß1a1/MBP-72-89 digested with NcoI and XhoI, to produce a plasmid encoding the ß1a1/CM-2 covalent construct.

Protein expression was tested in a number of different *E. coli* strains, including a thioredoxin reductase mutant which allows disulfide bond formation in the cytoplasm (Derman et al., 1993). With such a small molecule, it became apparent that the greatest yield of material could be readily obtained from inclusion bodies, refolding the protein after solubilization and purification in buffers containing 6M urea. Accordingly, *E. coli* strain BL21(DE3) cells were transformed with the pET21d+ construct containing the ß1α1-encoding sequence. Bacteria were grown in one liter cultures to mid-logarithmic phase (OD_{600} = 0.6-0.8) in Luria-Bertani (LB) broth containing carbenicillin (50 µg/ml) at 37°C. Recombinant protein production was induced by addition of 0.5 mM isopropyl ß-D-thiogalactoside (IPTG). After incubation for 3 hours, the cells were centrifuged and stored at -80°C before processing. All subsequent manipulations of the cells were at 4°C. The cell pellets were resuspended in ice-cold PBS, pH 7.4, and sonicated for 4 x 20 seconds with the cell suspension cooled in a salt/ice/water bath. the cell suspension was then centrifuged, the supernatant fraction was poured off, the cell pellet resuspended and washed three times in PBS and then resuspended in 20 mM ethanolamine/6 M urea, pH 10, for four hours. After centrifugation, the supernatant containing the solubilized recombinant protein of interest was collected and stored at 4°C until purification. Recombinant ß1α1 construct was purified and concentrated by FPLC ion-exchange chromatography using Source 30Q anion-exchange media (Pharmacia Biotech, Piscataway, NJ) in an XK26/20 column (Pharmacia Biotech), using a step gradient with 20 mM ethanolamine/6M urea/1M NaCl, pH 10. The homogeneous peak of the appropriate size was collected, dialyzed extensively against PBS at 4°C, pH 7.4, and concentrated by centrifugal ultrafiltration with Centricon-10 membranes (Amicon, Beverly, MA). The dialysis step, which removed the urea from the protein preparation and reduced the final pH, resulted in spontaneous re-folding of the expressed protein. For purification to homogeneity, a finish step used size exclusion chromatography on Superdex 75 media (Pharmacia Biotech) in an HR16/50 column (Pharmacia Biotech). The final yield of purified protein varied between 15 and 30 mg/L of bacterial culture.

Conformational integrity of the molecules was demonstrated by the presence of a disulfide bond between cysteines β15 and β79 as detected on gel shift assay, and the authenticity of the purified protein was verified using the OX-6 monoclonal antibody specific for RT1B by Western Blotting (data not shown). Circular dichroism (CD) reveals that the β1α1 molecules have highly

production after stimulation with antigen in the presence of APCs (irradiated Lewis rat thymocytes) and then subcloned at limiting dilution. The A1 hybridoma secretes IL-2 when stimulated in the presence of APCs with whole Gp-BP or Gp-BP-69-89 peptide, which contains the minimum epitope, MBP-72-89.

Two color immunofluorescent analysis was performed on a FACScan instrument (Becton Dickinson, Mountain View, CA) using CellQuest™ software. Quadrants were defined using non-relevant isotype matched control antibodies. ß1α1 molecules with and without loaded peptide were incubated with the A1 hybridoma (10 μM ß1α1/peptide) for 17 hours, 4°C, washed three times, stained with fluorochrome (FITC or PE) conjugated antibodies specific for rat class II (OX6-PE), and TCR Vß8.2 (PharMingen, San Diego, CA) for 15 minutes at room temperature, and analyzed by flow cytometry. The CM-2 cell line was blocked for one hour with unconjugated OX6, washed and then treated as the A1 hybridoma. Staining media was PBS, 2% fetal bovine serum, 0.01% azide.

Results

Epitope-specific binding was evaluated by loading the β1α1 molecule with various peptides and incubating β1α1/peptide complexes with the A1 hybridoma that recognizes the MBP-72-89 peptide (Burrows et al., 1997), or with a cardiac myosin CM-2-specific cell line. As is shown in Fig. 3A, the β1α1 construct loaded with MBP-69-89 peptide (β1α1/MBP-69-89) specifically bound to the A1 hybridoma, with a mean fluorescence intensity (MFI) of 0.8×10^3 Units, whereas the β1α1 construct loaded with CM-2 peptide (β1α1/CM-2) did not stain the hybridoma. Conversely, β1α1/CM-2 specifically bound to the CM-2 line, with a MFI of 1.8×10^3 Units, whereas the β1α1/MBP-69-89 complex did not stain the CM-2 line (Fig. 3B). The β1α1 construct without exogenously loaded peptide does not bind to either the A1 hybridoma (Fig. 3A) nor the CM-2 line (data not shown). Thus, bound epitope directed the specific binding of the β1α1/peptide complex.

Example 3: **<u>β1α1 Molecules Conjugated With A Fluorescent Label</u>**

To avoid using a secondary antibody for visualizing the interaction of β1α1/peptide molecules with TCR (such as OX-6, used above), a β1α1 molecules directly conjugated with a chromophore was produced. The Alexa-488™ dye (A488; Molecular Probes, Eugene, OR) has a spectra similar to fluorescein, but produces protein conjugates that are brighter and more photo-stable than fluorescein conjugates. As is shown in figure 4, A488-conjugated β1α1 (molar ratio dye/protein = 1), when loaded with MBP-69-89, bound to the A1 hybridomas (MCI = 300 Units), whereas empty β1α1 did not.

Example 4: **<u>β1α1 Molecules Inhibit Epitope-Specific T-cell Proliferation *In Vitro*</u>**

T-cell proliferation assays were performed to evaluate the effect of the constructs on T cell activation.

Materials and methods

Spinal cord mononuclear cells were isolated by a discontinuous percol gradient technique and counted as previously described (Bourdette et al., 1991). The cells were stained with fluorochrome (FITC or PE) conjugated antibodies specific for rat CD4, CD8, CD11b, CD45ra, TCR Vß8.2 and CD134 (PharMingen, San Diego, CA) for 15 min at room temperature and analyzed by flow cytometry. The number of positive staining cells per spinal cord was calculated by multiplying the percent staining by the total number of cells per spinal cord. Control and ß1α1/MBP-69-89 protected rats were sacrificed at peak and recovery of clinical disease, spinal cords were dissected and fixed in 10% buffered formalin. The spinal cords were paraffin-embedded and sections were stained with luxol fast blue-periodic acid schiff-hematoxylin for light microscopy.

Results

Intravenous injection (i.v.) of 300 µg of the β1α1/MBP-69-89 complex in saline on days 3, 7, 9, 11, and 14 after injection of MBP or MBP-69-89 peptide in CFA suppressed the induction of clinical (Fig. 6 and Table 3) and histological (not shown) signs of EAE. Injection of as little as 30 µg of the ß1α1/MBP-69-89 complex following the same time course was also effective, completely suppressing EAE in 4 of 6 rats, with only mild signs in the other 2 animals. All of the control animals that were untreated, that received 2 µg MBP-69-89 peptide alone (the dose of free peptide contained in 30 µg of the complex), or that received 300 µg of the empty ß1α1 construct developed a comparable degree of paralytic EAE (Table 2). Interestingly, injection of 300 µg of a control ß1α1/CM-2 peptide complex produce a mild (about 30%) suppression of EAE (Fig. 6 and Table 2). In parallel with the course of disease, animals showed a dramatic loss in body weight (Fig. 6), whereas animals treated with the ß1α1/MBP-69-89 complex showed no significant loss of body weight throughout the course of the experiment.

infiltration of activated inflammatory cells into the CNS. Mononuclear cells were isolated from the spinal cords of control and protected animals at peak and recovery of clinical disease and examined by FACS analysis. The total number of mononuclear cells isolated from spinal cords of control animals at peak of clinical disease (day 14) was 40-fold higher than from protected animals evaluated at the same time point (Table 3). Moreover, protected animals had 72% fewer activated (OX40+), Vß8.2+ T cells in the spinal cord when compared to control animals (Table 3). CD4+ and CD8+ T cells, macrophages and B cell numbers were also significantly reduced in protected animals (not shown). The number of mononuclear cells isolated after recovery from EAE was reduced 4.5-fold in protected animals (0.64×10^5 cells/spinal cord) compared to control animals (2.9×10^5 cells/spinal cord). Protected animals also had 10-fold fewer activated (OX40+), Vß8.2+ T cells in the spinal cord than control animals after recovery from disease.

Treatment with ß1α1/MBP-69-89 complex specifically inhibited the delayed-type hypersensitivity (DTH) response to MBP-69-89. As shown in Fig. 8A, changes in ear thickness 24 hours after challenge with PPD were uneffected by in animals treated with ß1α1 or ß1α1 loaded with peptides. However, as is shown in Fig. 8B, while animals treated with ß1α1 alone or complexed with CM-2 had no effect on the DTH response, animals treated with the ß1α1/MBP-69-89 complex showed a dramatic inhibition of the DTH response to MBP-69-89.

Treatment of EAE with the ß1α1/MBP-69-89 complex also produced an inhibition of lymph node (LN) T cell responses. As is shown in Fig. 9, LN cells from rats treated with the suppression protocol (Fig. 6) were inhibited 2-4 fold in response to MBP or the MBP-69-89 peptide compared to control rats. This inhibition was antigen specific, since LN T cell responses to PPD (stimulated by the CFA injection) were the same in treated and control groups. T cell responses tested in rats treated after disease onset (Fig. 7) were also inhibited, in an IL-2 reversible manner. LN cell responses to MBP and MBP-69-89 peptide were optimal (S.I = 4-5X) at low antigen (Ag) concentrations (4 µg/ml), and could be enhanced 2-fold with additional IL-2. In contrast, responses were inhibited in treated rats, with optimal LN cell responses (±3X) requiring higher Ag concentrations (20-50 µg/ml). However, in the presence of IL-2, responses could be restored to a level comparable to control rats (S.I. = 6-11X) without boosting Ag concentrations.

Discussion

The following Examples illustrate the efficacy of the two-domain MHC molecules. While the experimental details concern the MHC class II ß1α1 polypeptides, it will be appreciated that these data fully support application of MHC class I α1α2 polypeptides.

In the presented Examples, polypeptides comprising the MHC class II ß1 and α1 domains are described. These molecules lack the α2 domain, the ß2 domain known to bind to CD4, and transmembrane and intra-cytoplasmic sequences. The reduced size and complexity of the ß1α1 construct permits expression and purification of the molecules from bacterial inclusion bodies in high yield. The ß1α1 molecules are shown to refold in a manner that allows binding of allele-specific

From a drug engineering and design perspective this prototypic molecule represents a major breakthrough. The demonstrated biological efficacy of the ß1α1/MBP-69-89 complex in EAE raises the possibility of using this construct as a template for engineering human homologs for treatment of autoimmune diseases such as multiple sclerosis, that likely involves inflammatory T cells directed at CNS proteins. One candidate molecule would be HLA-DR2/MBP-84-102, which includes both the disease-associated class II allele and a known immunodominant epitope that has been reported to be recognized more frequently in MS patients than controls. However, because of the complexity of T cell response to multiple CNS proteins and their component epitopes, it is likely that a more general therapy may require a mixture of several MHC/Ag complexes. The precision of inhibition induced by the novel ß1α1/MBP-69-89 complex reported herein represents an important first step in the development of potent and selective human therapeutic reagents. With this new class of reagent, it may be possible to directly quantify the frequency and prevalence of T cells specific for suspected target autoantigens, and then to selectively eliminate them in affected patients. Through this process of detection and therapy, it may then be possible for the first time to firmly establish the pathogenic contribution of each suspected T cell specificity.

Having illustrated and described the principles of synthesizing two domain class II β1α1 and class I α1α2 molecules and the methods of using such molecules, it will be apparent to one skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. We claim all modifications coming within the spirit and scope of the claims presented herein.

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11. The recombinant polypeptide according to claim 10, wherein the polypeptide further comprises an antigenic determinant associated with the polypeptide by covalent or non-covalent interaction.

12. The recombinant polypeptide according to claim 11 wherein the antigenic determinant is covalently linked to the amino terminus of the β1 domain.

13. The recombinant polypeptide according to claim 10 wherein the polypeptide further comprises a detectable marker or toxic moiety.

14. A recombinant polypeptide according to claim 1 wherein the first domain is a mammalian MHC class I α1 domain and the second domain is a mammalian MHC class I α2 domain.

15. A polypeptide comprising covalently joined α1 and α2 domains of a mammalian MHC class I molecule wherein the amino terminus of the α2 domain is covalently linked to the carboxy terminus of the α1 domain, and wherein the polypeptide does not include an MHC class I α3 domain.

16. The polypeptide according to claim 15, wherein the polypeptide further comprises an antigenic determinant associated with the polypeptide by covalent or non-covalent interaction.

17. The polypeptide according to claim 16 wherein the antigenic determinant is covalently linked to the amino terminus of the α1 domain.

18. The recombinant polypeptide according to claim 15 wherein the polypeptide further comprises a detectable marker or toxic moiety.

19. A nucleic acid molecule encoding a polypeptide according to claim 1.

20. A transgenic cell including a nucleic acid molecule according to claim 19.

21. A nucleic acid expression vector comprising a nucleic acid molecule according to claim 19.

22. A recombinant nucleic acid molecule, comprising first, second and third regions represented by the formula Pr-B-A, wherein:

Pr is a promoter sequence;

combining the biological sample with a recombinant polypeptide comprising covalently linked α1 and β1 domains of a mammalian MHC class II molecule wherein the carboxy terminus of the β1 domain is covalently linked to the amino terminus of the α1 domain, and further comprising the specified antigen bound in a peptide binding groove formed by said α1 and β1 domains; and

detecting or quantifying the presence of specific binding of the recombinant polypeptide with said T-cells.

27. A method for separating T-cells having a receptor specific for a specified antigen from a mixture of cells, comprising:

combining the cell mixture with a recombinant polypeptide comprising covalently linked α1 and β1 domains of a mammalian MHC class II molecule wherein the carboxy terminus of the β1 domain is covalently linked to the amino terminus of the α1 domain, and further comprising the specified antigen bound in a peptide binding groove formed by said α1 and β1 domains; and

separating those cells bound to the recombinant polypeptide from unbound cells.

28. A method for detecting or quantifying in a biological sample the presence of T-cells having a receptor specific for a specified antigen, comprising:

combining the biological sample with a polypeptide comprising covalently linked α1 and α2 domains of a mammalian MHC class I molecule wherein the carboxy terminus of the α1 domain is covalently linked to the amino terminus of the α2 domain, wherein the polypeptide does not include an α3 domain of a mammalian MHC class I molecule and wherein the polypeptide further comprises the specified antigen bound in a peptide binding groove formed by said α1 and α2 domains; and

detecting or quantifying the presence of specific binding of the recombinant polypeptide with said T-cells.

29. A method for separating T-cells having a receptor specific for a specified antigen from a mixture of cells, comprising:

combining the cell mixture with a polypeptide comprising covalently linked α1 and α2 domains of a mammalian MHC class I molecule wherein the carboxy terminus of the α1 domain is covalently linked to the amino terminus of the α2 domain, wherein the polypeptide does not include an α3 domain of a mammalian MHC class I molecule and wherein the polypeptide further comprises the specified antigen bound in a peptide binding groove formed by said α1 and α2 domains; and

separating those cells bound to the recombinant polypeptide from unbound cells.

30. A pharmaceutical composition comprising a polypeptide according to claim 1 and a pharmaceutically acceptable carrier.

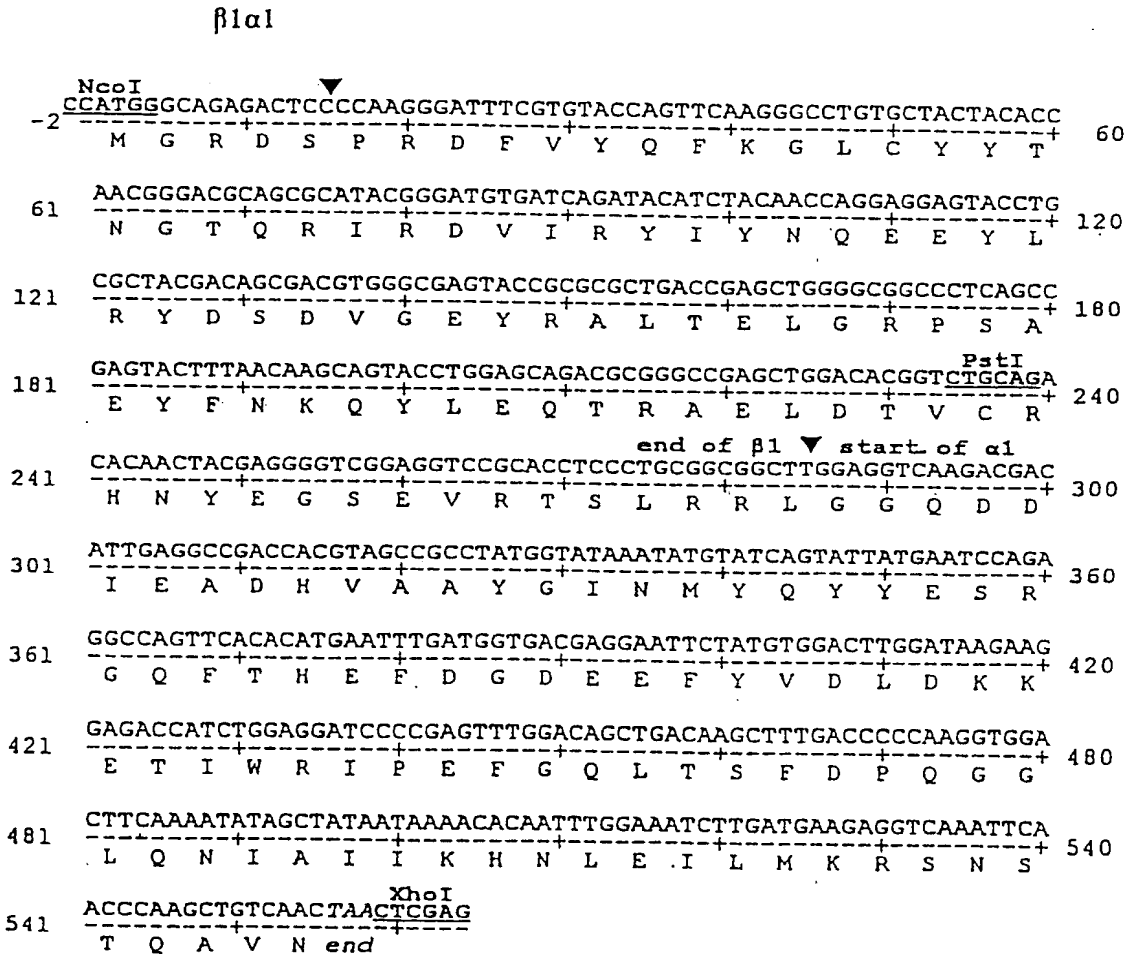
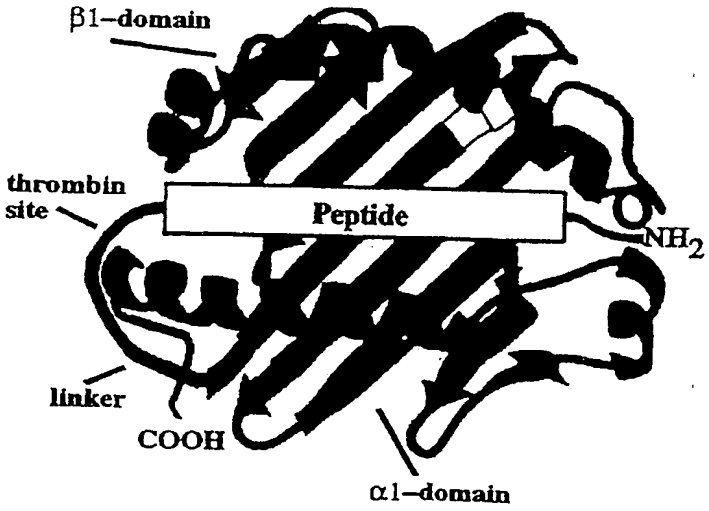


FIG. 1A

a. RT1.B



b. β1α1/peptide

FIG. 2

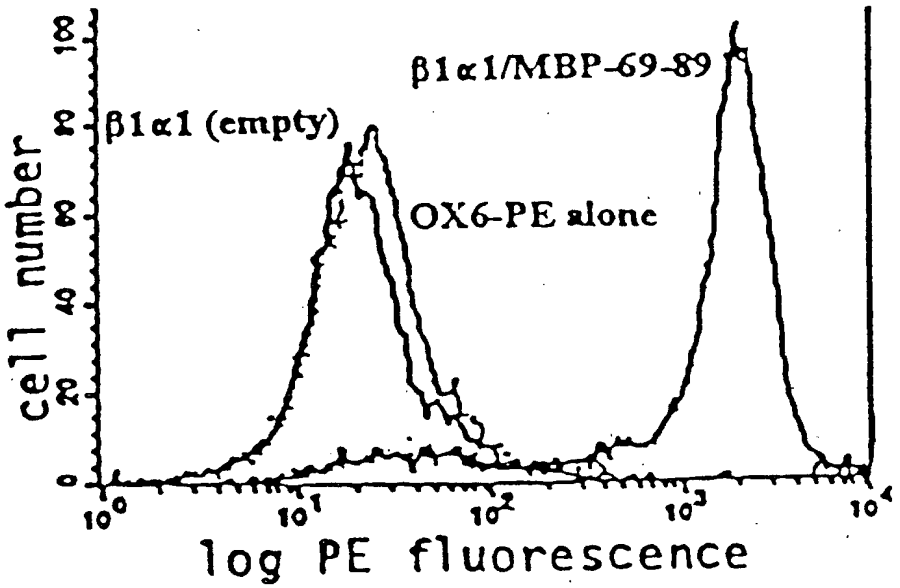
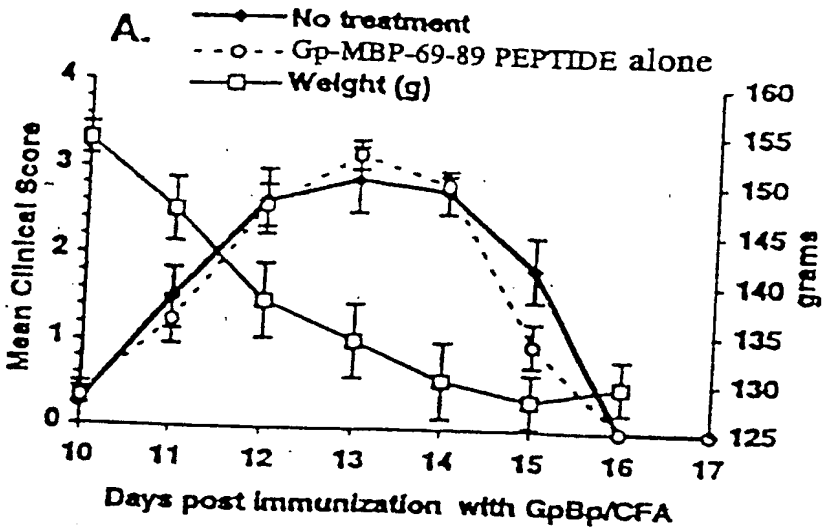


FIG. 4



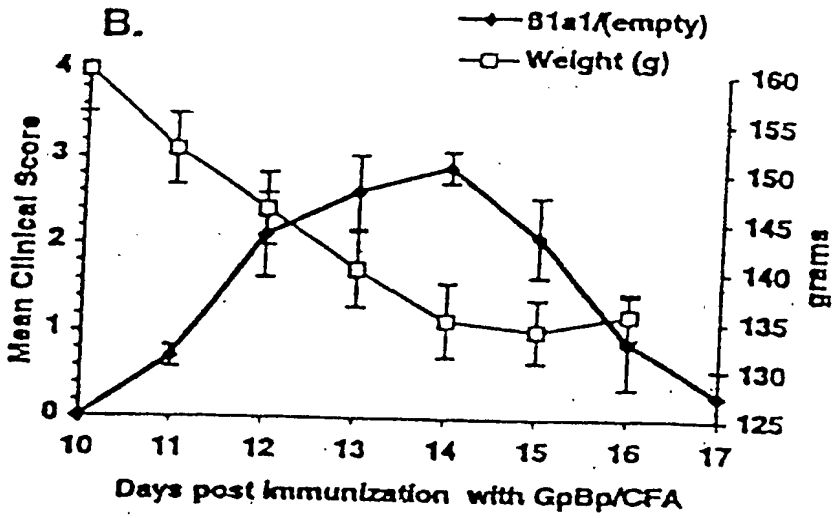


FIG. 6A

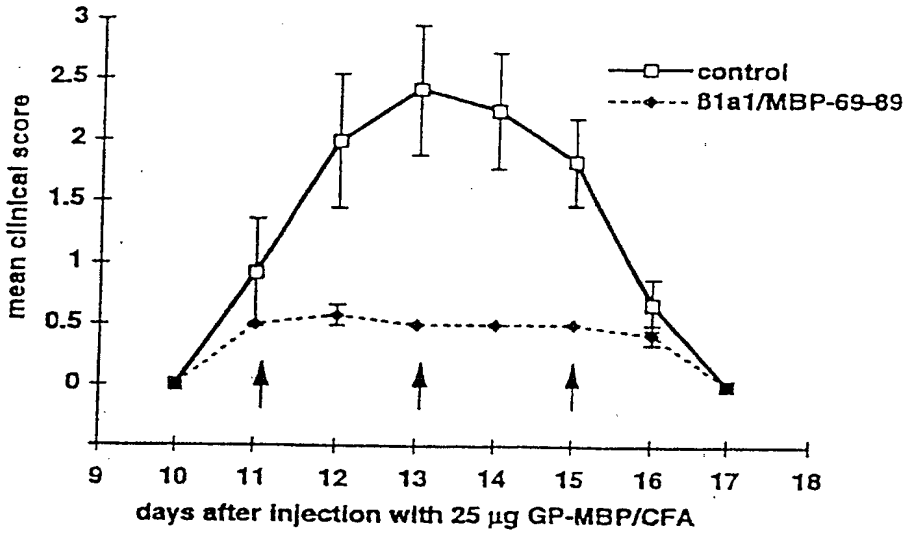


FIG. 7

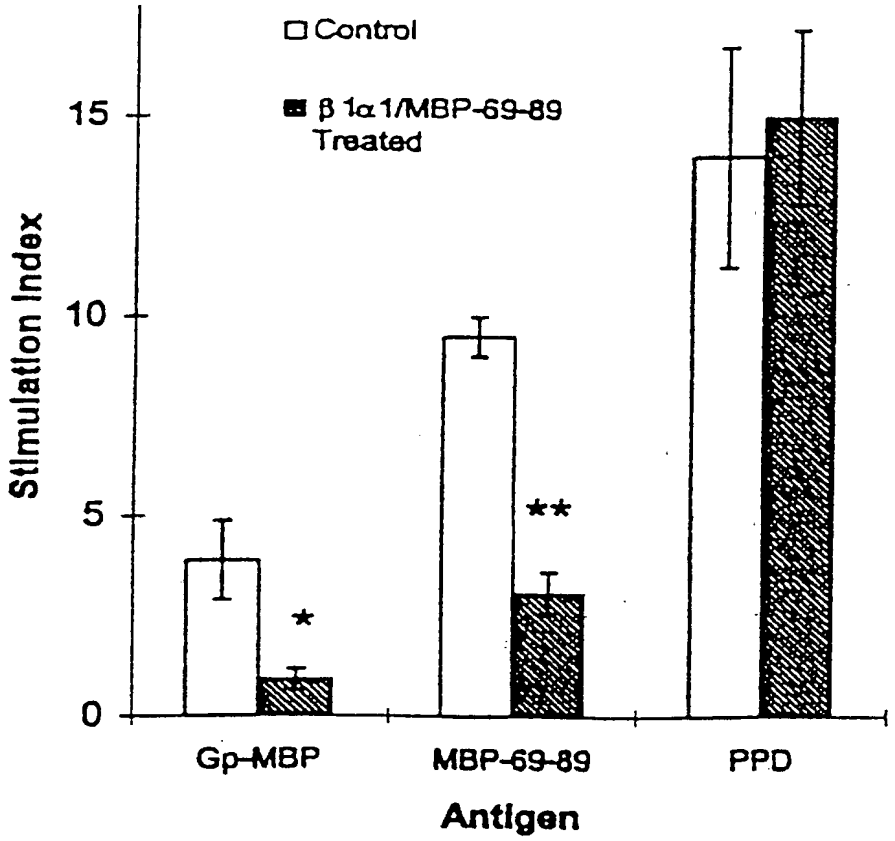


FIG. 9

β1 domain:
ARG4-PRO5-TRP6-PHE7-LEU8-GLU9-TYR10-CYS11-LYS12-SER13-GLU14-CYS15-
HIS16-PHE17-TYR18-ASN19-GLY20-THR21-GLN22-ARG23-VAL24-ARG25-LEU26-
LEU27-VAL28-ARG29-TYR30-PHE31-TYR32-ASN33-LEU34-GLU35-GLU36-ASN37-
LEU38-ARG39-PHE40-ASP41-SER42-ASP43-VAL44-GLY45-GLU46-PHE47-ARG48-
ALA49-VAL50-THR51-GLU52-LEU53-GLY54-ARG55-PRO56-ASP57-ALA58-GLU59-
ASN60-TRP61-ASN62-SER63-GLN64-PRO65-GLU66-PHE67-LEU68-GLU69-GLN70-
LYS71-ARG72-ALA73-GLU74-VAL75-ASP76-THR77-VAL78-CYS79-ARG80-HIS81-
ASN82-TYR83-GLU84-ILE85-PHE86-ASP87-ASN88-PHE89-LEU90-VAL91-PRO92-
ARG93-ARG94-VAL95

α1 domain:
GLU3-GLU4-HIS5-THR6-ILE7-ILE8-GLN9-ALA10-GLU11-PHE12-TYR13-LEU14-
LEU15-PRO16-ASP17-LYS18-ARG19-GLY20-GLU21-PHE22-MET23-PHE24-ASP25-
PHE26-ASP27-GLY28-ASP29-GLU30-ILE31-PHE32-HIS33-VAL34-ASP35-ILE36-
GLU37-LYS38-SER39-GLU40-THR41-ILE42-TRP43-ARG44-LEU45-GLU46-GLU47-
PHE48-ALA49-LYS50-PHE51-ALA52-SER53-PHE54-GLU55-ALA56-GLN57-GLY58-
ALA59-LEU60-ALA61-ASN62-ILE63-ALA64-VAL65-ASP66-LYS67-ALA68-ASN69-
LEU70-ASP71-VAL72-MET73-LYS74-GLU75-ARG76-SER77-ASN78-ASN79-THR80-
PRO81-ASP82-ALA83-ASN84

FIG. 10B

SEQUENCE LISTING

<110> Burrows et al.

<120> Recombinant MHC molecules useful for manipulation of antigen-specific T-Cells

<130> 50963

<140>
<141>

<150> 60/064,552
<151> 1997-09-16

<150> 60/064,555
<151> 1997-10-10

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     1               5                  10                  15

ctg tgc tac tac acc aac ggg acg cag cgc ata cgg gat gtg atc aga    95
Leu Cys Tyr Tyr Thr Asn Gly Thr Gln Arg Ile Arg Asp Val Ile Arg
                20                  25                  30

tac atc tac aac cag gag gag tac ctg cgc tac gac agc gac gtg ggc   143
Tyr Ile Tyr Asn Gln Glu Glu Tyr Leu Arg Tyr Asp Ser Asp Val Gly
            35                  40                  45

gag tac cgc gcg ctg acc gag ctg ggg cgg ccc tca gcc gag tac ttt   191
Glu Tyr Arg Ala Leu Thr Glu Leu Gly Arg Pro Ser Ala Glu Tyr Phe
        50                  55                  60

aac aag cag tac ctg gag cag acg cgg gcc gag ctg gac acg gtc tgc   239
Asn Lys Gln Tyr Leu Glu Gln Thr Arg Ala Glu Leu Asp Thr Val Cys
    65                  70                  75

aga cac aac tac gag ggg tcg gag gtc cgc acc tcc ctg cgg cgg ctt   287
Arg His Asn Tyr Glu Gly Ser Glu Val Arg Thr Ser Leu Arg Arg Leu
80                  85                  90                  95

gga ggt caa gac gac att gag gcc gac cac gta gcc gcc tat ggt ata   335
Gly Gly Gln Asp Asp Ile Glu Ala Asp His Val Ala Ala Tyr Gly Ile
                100                 105                 110

aat atg tat cag tat tat gaa tcc aga ggc cag ttc aca cat gaa ttt   383
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<213> Artificial Sequence

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1 5 10 15

aac cca gtg gtg cac ttc gga ggt gga ggc tca cta gtg ccc cga ggc 95
Asn Pro Val Val His Phe Gly Gly Gly Gly Ser Leu Val Pro Arg Gly
20 25 30

tct gga ggt gga ggc tcc 113
Ser Gly Gly Gly Gly Ser
35

<210> 4
<211> 37
<212> PRT
<213> Artificial Sequence

<400> 4
Met Gly Arg Asp Ser Pro Gln Lys Ser Gln Arg Thr Gln Asp Glu Asn
1 5 10 15

Pro Val Val His Phe Gly Gly Gly Gly Ser Leu Val Pro Arg Gly Ser
20 25 30

Gly Gly Gly Gly Ser
35

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: PCR primer

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<210> 10
<211> 30
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<211> 98
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<213> Artificial Sequence

<220>
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actgcagttc cagtttggag tctctgccca tggtaata 98

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Arg Gly Glu Pro Arg Phe Ile Ala Val Gly Tyr Val Asp Asp Thr Gln
20 25 30

Phe Val Arg Phe Asp Ser Asp Ala Ala Ser Pro Arg Thr Glu Pro Arg
35 40 45

Pro Pro Trp Ile Glu Gln Glu Gly Pro Glu Tyr Trp Asp Arg Asn Thr
50 55 60

Gln Ile Phe Lys Thr Asn Thr Gln Thr Tyr Arg Glu Asn Leu Arg Ile
65 70 75 80

Ala Leu Arg Tyr Tyr Asn Gln Ser Glu Ala Gly Ser His Ile Ile Gln
85 90 95

Arg Met Tyr Gly Cys Asp Leu Gly Pro Asp Gly Arg Leu Leu Arg Gly
100 105 110

His Asp Gln Ser Ala Tyr Asp Gly Lys Asp Tyr Ile Ala Leu Asn Glu
115 120 125

Gly Thr Gln Arg Val Arg Leu Leu Val Arg Tyr Phe Tyr Asn Leu Glu
20 25 30

Glu Asn Leu Arg Phe Asp Ser Asp Val Gly Glu Phe Arg Ala Val Thr
35 40 45

Glu Leu Gly Arg Pro Asp Ala Glu Asn Trp Asn Ser Gln Pro Glu Phe
50 55 60

Leu Glu Gln Lys Arg Ala Glu Val Asp Thr Val Cys Arg His Asn Tyr
65 70 75 80

Glu Ile Phe Asp Asn Phe Leu Val Pro Arg Arg Val Glu Glu His Thr
85 90 95

Ile Ile Gln Ala Glu Phe Tyr Leu Leu Pro Asp Lys Arg Gly Glu Phe
100 105 110

Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Ile Glu Lys
115 120 125

Ser Glu Thr Ile Trp Arg Leu Glu Glu Phe Ala Lys Phe Ala Ser Phe
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Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu
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Asp Val Met Lys Glu Arg Ser Asn Asn Thr Pro Asp Ala Asn
165 170

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<212> PRT
<213> Rattus sp.

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20 25 30

Ile Tyr Asn Gln Glu Glu Tyr Leu Arg Tyr Asp Ser Asp Val Gly Glu
35 40 45

Tyr Arg Ala Leu Thr Glu Leu Gly Arg Pro Ser Ala Glu Tyr Trp Asn
50 55 60

Ser Gln Lys Gln Tyr Leu Glu Gln Thr Arg Ala Glu Leu Asp Thr Val
65 70 75 80

Cys Arg His Asn Tyr Glu Gly Ser Glu Val Arg Thr Ser Leu Arg Arg
85 90 95

Leu Ala Asp His Val Ala Ala Tyr Gly Ile Asn Met Tyr Gln Tyr Tyr
100 105 110

Glu Ser Arg Gly Gln Phe Thr His Glu Phe Asp Gly Asp Glu Glu Phe
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<210> 28
<211> 95
<212> DNA
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<220>
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ggtgcacttc ggaggtggag gctcactagt gcccc 95

<210> 29
<211> 94
<212> DNA
<213> Artificial Sequence

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ggctcttctg tggggagtct ctgcccatgg taat 94

<210> 30
<211> 19
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: artificial peptide

<400> 30
Gly Ser Leu Pro Gln Lys Ser Gln Arg Thr Gln Asp Glu Asn Pro Val
1 5 10 15

Val His Phe

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/18244

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/7.21, 7.24, 235, 320.1 455; 530/351, 403, , 413, , 402; 536/23.4